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TestAmerica, Inc.

Standard Operating Procedure

Analyte or Suite: Nitrogen, Free Ammonia

Methodology: Automated Phenate Colorimetric

Reference: EPA-600/4-79-020 Revised March 1983, Method 350.1,

Revision # 3 Date revised: 15 October 1999

Approvals:

Divisional Manager

Quality Assurance Coordinator

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1. Introduction and Scope

1.1. General.

Preservation: 1 mL H_2SO_4 per liter, refrigerate at 4°C

Container: 250 mL plastic or glass

Minimum sample volume: 50 mL (free NH_3)

Holding Time: 28 days

Range of Test: 0.02 to 1.00 mg/L (free NH_3)

Nominal Reporting Limit: 0.05 mg/L (free NH_3)

Method No.(s) and References:
350.1 EPA 600/4-79-020, Revised March 1983

Regulatory Limits: Not Applicable

Wavelength Setting: 660 nm

1.2. This SOP can be used to determine the concentration of inorganic free ammonia nitrogen in water and wastewater. In waters and wastewaters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, nitrate, nitrite, ammonia, and organic nitrogen. All of these forms of nitrogen, as well as nitrogen gas (N_2), are biochemically interconvertible and are components of the nitrogen cycle. They are of interest for many reasons.

1.3. In the following paragraphs, organic nitrogen is referred to as organic N, nitrate nitrogen as NO_3^--N, nitrite nitrogen as NO_2^--N, and ammonia nitrogen as NH3-N.

1.4. Ammonia is a colorless gas with a pungent odor; it is highly soluble in water. When ammonia dissolves in water, the equilibrium may be expressed by the following equation:

$$NH_3 + H_2O \text{ <--> } (NH_4^+) + (OH^-)$$

With increasing temperature or pH, this equilibrium is shifted toward unionized ammonia (NH_3). NH_3 concentration decreases with increasing ionic strength or salinity. Ammonia adsorbs to soil particles, especially in alkaline water, and does not leach readily. Thus, ammonia concentration is normally low in non-polluted natural waters.

1.5. Ammonia is the primary end product of the anaerobic decomposition of organic matter by heterotrophic bacteria and by hydrolysis of urea. Ammonia is present naturally in surface and

wastewaters. Possible pollution is indicated by higher than normal amounts of ammonia being present. Ammonia concentrations encountered in waters vary from less than 10 ug ammonia nitrogen/L in some natural surface and groundwaters to more than 30 mg ammonia nitrogen/L in wastewaters.

1.6. Ammonia is an important pollutant in raw waters for public water supply systems because its reaction with chlorine forms mono- and dichloramines, which have less disinfecting capacity than free chlorine. Until the ammonia is totally oxidized, there will be practically no free chlorine residual.

1.7. Unionized ammonia (NH_3), the concentration of which is pH and temperature dependent, can be toxic to fish and aquatic animals. The pH of most natural waters is such that the equilibrium favors NH_4^+, but in alkaline waters, the concentration of NH_3 can reach toxic levels. Studies have shown that the lethal concentration of ammonia for a variety of fish species is from 0.2 mg/L NH_3 (for trout) to 2.0 mg/L NH_3 (for carp). The USEPA has set a criteria of 0.02 mg/L NH_3 for freshwater aquatic life.

1.8. Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems, and natural waters. Nitrite can enter a water supply system through its use as a corrosion inhibitor in industrial process water. Nitrite is the actual etiologic agent of methemoglobinemia. Nitrous acid, which is formed from nitrite in acidic solution, can react with secondary amines (RR'NH) to form nitrosamines (RR'N-NO), many of which are known to be carcinogens. The toxicologic significance of nitrosation reactions in vivo and in the natural environment is the subject of much current concern and research.

1.9. Ammonia determination in this automated methodology follows the Berthelot Reaction. The formation of a blue colored compound closely related to indophenol occurs when a solution of ammonium salt is added to sodium phenoxide followed by sodium hypochlorite. An EDTA solution is added to the sample stream to eliminate the precipitation of calcium and magnesium hydroxides. Sodium nitroprusside is added to intensify the blue/green color produced. The intensity of the resultant color compound is directly related to the concentration of ammonia present and is read automatically in the Technicon Traacs 800 autoanalyzer at 660 nm.

1.10. Your ability to follow this method according to the specifications herein will affect your performance evaluations, the quality of data produced for this application, your section's productivity, and the laboratory's profitability.

2. Summary of Method

2.1. In the colorimetric measurement, the ammonia ion reacts with sodium phenate followed by sodium hypochlorite in the presence of sodium nitroprusside to produce an intense blue/green compound closely related to indophenol. An EDTA solution is added to the sample stream to prevent precipitation of calcium and magnesium hydroxides. This intense blue/green compound is then read colorimetrically at 660 nm in the Technicon Traacs 800 Autoanalyzer.

3. Safety

Each employee is directly responsible for complete awareness of all health hazards associated with every chemical that he/she uses. The employee must be aware of these hazards, and all associated protective wear and spill clean-up procedures PRIOR TO THE USE of any chemical. In all cases, both the applicable MSDS and supervisor or Safety Officer should be consulted. The employee should comply with all safety policies as presented in the TestAmerica Safety Manual. The bottle labels also provide important information that must be noted. If you have any questions, consult your supervisor or safety officer.

Personnel performing this procedure may be working with flammables, poisons, toxins, carcinogens, teratogens, mutagens, and biohazards. In particular, approved gloves, safety glasses, and labcoats must be worn, and solvents will be handled in ventilated hoods, in addition to other measures prescribed by the Division. It should be noted that samples must be handled with as much care as any of the materials used in this method due to the unknown nature of their composition.

4. Reagents and Materials

4.1. Apparatus.

The following apparatus is recommended for performing this procedure. Equivalent items can be used. An item can be considered equivalent if with its use, the analytical and QA/QC requirements in this SOP can be met.

4.1.1. Bran & Luebbe Technicon Traacs 800 Autoanalyzer with 660 nm filter, 0.5 x 10 mm flowcell.

4.1.2. Eppendorf 500 uL autopipetter with appropriate pipet tips.

4.1.3. Eppendorf 1000 uL autopipetter with appropriate pipet tips.

4.1.4. Oxford macroset autopipetter with appropriate pipet tips.

4.2. Reagents.

The following reagents are required to perform this procedure. When instructions are given on how to prepare a specific volume of a reagent, larger or smaller volumes can be prepared as needed so long as the final concentrations remain the same. Any other deviation from the reagents used in this SOP could be detrimental to the quality of the data produced. Such deviations would have to be approved by the corporate technical support team and documented.

All reagents must be properly labeled with the reagent identification and concentration, date prepared, expiration date, initials of analyst, and applicable safety information.

4.2.1. Deionized water: Prepare by passing water through a mixed bed of cation and anion exchange resins or an equivalent source. Use deionized water for the preparation of all reagents, calibration standards, and dilution water.

4.2.2. Sodium Hydroxide, reagent grade: Purchased shelf life as listed by manufacturer or 1 year.

4.2.3. Phenol, liquefied, reagent grade (91.7 %): Purchased shelf life as listed by manufacturer or 1 year.

4.2.4. Brij-35, 30 % solution: Purchased shelf life as listed by manufacturer or 1 year. Available from Bran & Luebbe, catalog # T21-0110-06

4.2.5. Alkaline Phenol: Dissolve 24 g NaOH pellets in about 400 mL of DI water. Add 42 mL of liquefied phenol (about 90 %) and cool to room temperature. Dilute to 500 mL volumetrically with DI water. Store in an amber glass container. Shelf life is two weeks.

4.2.6. Disodium ethylenediamine-tetraacetate (EDTA): Purchased shelf life as listed by manufacturer or 1 year.

4.2.7. Disodium EDTA: Dissolve 0.5 g NaOH pellets in about 800 mL of DI water. Add 41.0 g disodium EDTA and mix until dissolved completely. Hint: This may require gentle heating to accomplish. Cool to room temperature and dilute to 1000 mL volumetrically with DI water. Add 3.0 mL Brij-35 and mix thoroughly. Store in plastic or glass container of 1 L capacity. Shelf life is variable. When baseline problems are noticed, remake the reagent.

4.2.8. Sodium Hypochlorite (Clorox, 5.25 % active): Purchased shelf life as listed by manufacturer or 1 year.

4.2.9. Sodium Hypochlorite solution: Dilute 82 mL of commercially available sodium hypochlorite (Clorox, 5.25 % active) to 100 mL with DI water and mix thoroughly. Store in 250 mL plastic container. Shelf life is 1 week.

4.2.10. Sodium Nitroprusside: Purchased shelf life as manufacturer listed or 1 year.

4.2.11. Sodium Nitroprusside: Dissolve 1.1 g sodium nitroprusside in about 600 mL of DI water, and dilute to 1000 mL volumetrically with DI water. Store in amber glass container. Shelf life is 1 month.

4.2.12. Sulfuric Acid, concentrated, reagent grade: Purchased shelf life as manufacturer listed or 1 year.

4.3. Standards.

The following standards are recommended for performing this procedure. The use of alternative standards will be allowed as long as they are of equal or greater quality and there is an associated improvement in efficiency, productivity, or cost. When instructions are given on how to prepare a specific volume of standard, larger or smaller volumes can be prepared as needed so long as the volumes used are properly documented.

4.3.1. Ammonium Chloride, reagent grade: Purchased shelf life as manufacturer listed or 1 year.

4.3.2. Stock ammonia calibrant solution (1000 mg/L): Dissolve 3.819 g ammonium chloride (NH_4Cl) in 800 mL of deionized water and dilute to 1000 mL volumetrically. Store in glass 1 L bottle. Shelf life is 1 year.

4.3.3. Stock ammonia alternate source solution (1000 mg/L): Dissolve 3.819 g ammonium chloride (NH_4Cl) in 800 mL of deionized water and dilute to 1000 mL volumetrically. Store in glass 1 L bottle. Shelf life is 1 year.

4.3.4. Working ammonia calibrant stock (10 mg/L): Dilute 10.0 mL of the stock ammonia calibrant solution (1000 mg/L) to 1000 mL volumetrically. Make fresh monthly. Store in glass bottle.

4.3.5. Working ammonia alternate source stock (10.0 mg/L): Dilute 10.0 mL of the stock ammonia alternate source solution (1000 mg/L) to 1000 mL volumetrically. Make fresh monthly. Store in glass 1 L bottle.

4.3.6. Prepare run calibrants weekly. Store in the refrigerator. See directions below for preparation guidelines:

mLs of 10 mg/L calibrant stock	Concentration mg/L
#1) 10.0 mL to 100 mL volume	1.00
#2) 5.0 mL to 100 mL volume	0.50
#3) 2.0 mL to 100 mL volume	0.20
#4) 10.0 mL of #1 to 100 mL volume	0.10
#5) 10.0 mL of #1 to 200 mL volume	0.05

Note: Before running, the calibrants are acidified with 2 drops of H_2SO_4 per 100 mL to matrix match the samples.

A generic formula is based upon the proportion, C1V1 = C2V2 where:

C1 = Concentration 1 C2 = Concentration 2
V1 = Volume 1 V2 = Volume 2

Example: $\frac{1000 \text{ mg/L stock standard} \times 1 \text{ mL}}{100 \text{ mL final volume}}$ = 10 mg/L standard

4.3.7. Prepare a mid-level ICV from the working alternate source ammonia stock (10.0 mg/L) to validate your original curve. Pipet 8.0 mL of the 10.0 mg/L working alternate source ammonia solution into a 100 mL volumetric flask and bring up to volume with deionized water. The concentration of the ICV will be 0.80 mg/L.

4.3.8. Recommended spiking solution: Use the 10 mg/L working calibrant solution in section 4.3.4. Pipet 0.5 mL of this solution into 10.0 mL of the sample to be spiked, and mix thoroughly. The value of the spike is 0.5 mg/L. Spikes are performed at the instrument for free ammonia.

INTERFERENCES

5.1. Interferences include turbidity, which may be removed by filtration. Samples exhibiting inherent color that absorbs in the photometric range of interest show interference. Hydroxides of calcium and magnesium that precipitate may be minimized by the addition of EDTA into the sample stream.

6. Analytical Procedures

6.1. Preservation and Handling.

6.1.1. Samples should arrive preserved with 1 mL H_2SO_4 per liter.

6.1.2. Samples should be collected in plastic or glass bottles of 250 mL size or larger for free ammonia.

6.1.3. Samples should be refrigerated at 4°C and analyzed within 28 days.

6.1.4. Liquid samples should be run neat and settled for free ammonia.

6.2. Instrument Calibration.

6.2.1. Preparation of standard curve: The data used in plotting a calibration curve will consist of five standards evenly distributed throughout the linear range of the method. This data must be collected under the same conditions as those that will exist during routine analyses. Prepare a standard curve by plotting the absorbance values of standards (y-axis) versus the corresponding concentrations (x-axis).

6.2.2. Calibration criteria - ALL of the following must be met for a successful calibration OR the manufacturer's calibration specifications shall be specified below and met:

6.2.2.1. A correlation coefficient of 0.995 or greater must be achieved using all calibration standards.

6.2.2.2. From the linear least squares model, back calculate the standards' values. These values must not differ from the theoretical standards' values by more than ± 10% for all standards except the lowest standard. The low standard must meet the criteria of not differing from the theoretical value by more than 20 %.

6.2.2.3. Verify the curve by analyzing an Initial Calibration Verification Standard (ICVS) and obtaining a value within ± 10% of the true value.

6.3. Sample Analysis.

6.3.1. Automated Spectrophotometric Determination.

Configure the Technicon Traacs 800 Autoanalyzer according to the specifics in the manufacturer's method # 780-86T, available from Bran & Luebbe, entitled Ammonia In Water and Wastewater. Set up the reagent-sequencing mantle following the manufacturer's instructions. Only the correct glassware, pump tubes, connectors, and flowcells should be used since they are parameter specific.

6.3.2. Set-up the analytical run according to the instrument manufacturers sequence rules and analyze all samples and QC against the daily calibration curve for ammonia.

7. Quality Control.

The following details the QC requirements which apply to this analysis. Each Quality Control Indicator (QCI) provides information pertaining to either instrument performance, method performance (including sample preparation), or individual sample performance. Our goal is to produce data of unquestionable quality. Always remember what purpose the QCI serves when

evaluating QCI results. Guidelines can be provided, and are provided, but they cannot take the place of a logistical, common-sense evaluation of the complete data set.

7.1. Method Detection Limits and Reporting Limits.

An MDL study, following the MDL SOP, must be done during initial method validation and then annually. If the analytical method is changed, an MDL study must be done again. Also, the calculated MDL must not exceed the reporting limit. The current nominal reporting limit for this parameter is 0.05 mg/L for free ammonia.

7.2. Method Validation Sample (MVS).

7.2.1. Definition and Use of MVS

The purpose of the MVS is to verify and demonstrate that the method and/or analyst is capable of generating precise and accurate analytical data. Method validation samples consist of four replicate aliquots of spiked deionized water prepared and analyzed in a manner identical to samples. The spike concentration should be at the mid range of the analysis. The samples should be prepared and analyzed in the same batch. They are used to validate new analyst and new instrument performance, and to validate changes in analytical equipment or techniques.

7.2.2. Frequency of MVS

Method validation must be repeated whenever a significant change in the method or instrumentation is made which could cause the previous MVS to become invalidated. Also, they will be routinely analyzed as part of training and certification of analysts newly performing the analysis.

7.2.3. Criteria for MVS

The average percent recovery should pass the interim acceptance criteria applied to the MS/MSD, which is 80-120%, and the relative standard deviation should be within $\pm$20%.

7.2.4. Corrective Action for MVS

If a problem is indicated, it must be identified and corrected, and if necessary, MVSs must be re-prepared and re-analyzed. If the problem involves only the instrument, the MVSs must be re-analyzed.

7.2.5. Documentation

Since the MVSs serve several purposes, results of the method validation should be filed either with individual analyst training records, method validation records, or with instrument validation records. How they are filed is dependent on the reason for the study being performed. An alternative would be to

file the results jointly and cross reference other files as appropriate. In either case, the data must be retrievable.

7.3. Calibration Curve.

7.3.1. Definition and Use of Calibration Curve

The purpose of a calibration curve is to relate instrument response to sample concentration. It also provides a way of verifying that the instrument response, over a predetermined concentration range, can be predicted using a mathematical equation. If the responses were erratic, there would be no accurate way to relate response to concentration. Curves should consist of data consisting of a blank and five standards . The concentrations of the standards should be distributed over the working range of the curve and they should represent the low, mid, and high points of the curve.

7.3.2. Frequency of Preparing Calibration Curve

When a daily curve system is used, the curve should be re-prepared if during the analytical run a CCV fails and corrective action is unsuccessful.

7.3.3. Criteria for Calibration Curve

Refer to the section in the SOP detailing instrument calibration.

7.3.4. Corrective Action for Calibration Curve

Since the calibration curve is used for calculating results for all samples and quality control indicators, an analyte cannot be reported from a run in which the calibration curve did not meet the criteria listed in the SOP. Perform any corrective actions necessary, and re-analyze the curve, the samples, and the quality control indicators.

Care should be taken when choosing the concentrations of the standards for the calibration curve. If the intercept is large or if the correlation coefficient is poor, then the concentrations of the standards used in relation to the detection limit and linear range should be carefully evaluated.

7.3.5. Documentation

Raw data associated with constructing a calibration curve should be retrievable. The calibration curve for each run is stored electronically in the Technicon Traacs 800 Autoanalyzer computer software.

7.4. Initial Calibration Verification Standard (ICVS).

7.4.1. Definition and Use of ICVS

The purpose of the ICVS is to verify that the standards used to make the curve were chemically pure, prepared properly, and that they have not degraded significantly since the time they were made. The ICVS should be obtained from a different source than the one used to prepare the standards used to construct the curve. The concentration of the ICVS should be at or above the mid point of the range of the analysis. This standard does not go through sample preparation stages.

7.4.2. Frequency of ICVS

Analyze an ICVS immediately following a calibration curve to verify the curve.

7.4.3. Criteria for ICVS

The percent recovery should be within ± 10% of the true value.

7.4.4. Corrective Action for ICVS

If the criteria for the ICVS cannot be met, re-evaluate the calibration curve to verify that all criteria have been met. Verify the acceptability of the source used for preparing the ICVS. Evaluate the concentration of the ICVS compared to the linear range of the analysis and the reporting limit. The concentration of the ICVS should be within the mid to upper range of the curve. If none of the above solves the problem, contact your supervisor before proceeding with the analysis.

7.4.5. Documentation

Record the percent recovery of the ICVS on the raw data printout or in the lab book.

7.5. Reagent Blank (RB) / Continuing Calibration Blank (CCB)

7.5.1. Definition and Use of Reagent Blank

The reagent blank is a deionized water blank that is subjected to the same conditions that a non-prepared sample undergoes. The reagent blank will determine if any contamination or any memory effects are occurring. Normally, a reagent blank is analyzed every time a CCVS is analyzed.

The reagent blank may contain background color inherent to the analytical procedure, which must be taken into account, during the analytical process.

7.5.2. Frequency of Reagent Blank

Analyze a minimum of one reagent blank at the beginning and one at the end of each analytical batch. Also, analyze a reagent blank after a minimum of every tenth sample.

7.5.3. Criteria for Reagent Blank

Acceptance criteria requires the reagent blank to be less than the reporting limit.

7.5.4. Corrective Action for Reagent Blank

Since the instrument/calculation is zeroed to the reagent blank, a reagent blank after the tenth sample or at the end of the run having a concentration greater than the reporting limit would indicate a contamination problem or possibly instrument drift. Determine the cause of the high reagent blank value, correct the problem, and re-analyze the samples following the last in control reagent blank/CCVS pair.

An in control reagent blank and an out of control preparation blank would be an indication of a contamination source within the sample preparation procedure.

7.5.5. Documentation

Record the concentration of the reagent blank on the raw data or in the lab book. Enter the reagent blank result into LABSYS2 into the blank entry.

7.6. Continuing Calibration Verification Standard (CCVS).

7.6.1. Definition and Use of CCVS

The continuing calibration verification standard is a mid standard that is subjected to the same conditions that a non-prepared sample undergoes. The CCVS will verify that the analytical system is in control with respect to the most recently run calibration curve. Normally, a CCVS is analyzed every time a reagent blank is analyzed.

7.6.2. Frequency of CCVS

Analyze a minimum of one CCVS at the end of each analytical batch. Also, analyze a CCVS after every tenth sample.

7.6.3. Criteria for CCVS

Acceptance criteria requires the percent recovery to be within 90-110% of the true value.

7.6.4. Corrective Action for CCVS

Rerun the CCVS, if it is still out of control, determine the cause, correct the problem, and re-analyze the samples following the last in control reagent blank/CCVS pair.

7.6.5. Documentation

Record the percent recovery of the CCVS on the raw data. Enter the percent recovery of the ending CCVS into LABSYS2 in the external standard entry.

7.7. Matrix Spike / Matrix Spike Duplicate (MS/MSD).

7.7.1 Definition and Use of MS/MSD

The matrix spike / matrix spike duplicate pair are two separate aliquots of sample which are spiked with known concentrations of analyte and subjected to the same conditions that a sample undergoes. The recommended spike concentration should be 20% of the top standard or equal the low or mid standard from a five point curve performed the day of the analysis. These data are generated to determine long-term precision and accuracy of the analytical method on various matrices. These data alone cannot be used to evaluate the precision and accuracy of individual samples except for the sample chosen for the MS/MSD analysis.

7.7.2. Frequency of MS/MSD

Analyze one MS/MSD pair per every 20 samples.

7.7.3. Criteria for MS/MSD

Advisory interim acceptance criteria requires the MS/MSD percent recovery to be within 75-125% and the relative percent difference to be less than 20.

7.7.4. Corrective Action for MS/MSD

No action is taken on out of control MS/MSD data alone to qualify an entire batch. Action taken must be weighed carefully since it may be difficult to determine if poor precision and/or accuracy is a result of sample non-homogeneity/uniqueness, method defects, or laboratory technique. However, the data may be used in conjunction with other QC criteria to determine the need for qualifying the data. If the MS/MSD data is outside acceptance limits, check percent recovery for the LCS. If the LCS is in control, the procedure is in control and the data is acceptable. Potentially, a matrix problem exists. Additional steps may be taken to determine the extent of the matrix interference. Refer to the Data Assessment and Review SOP for additional details.

If the concentration of an analyte in the client sample is >4x the level of the spike, then the spiking level is insignificant to the sample and skewed spike recoveries may result. This is not unexpected.

If an MS/MSD sample is diluted and the concentration of the spiked sample meets the above conditions, then the spike may be diluted out. This also is not unexpected.

7.7.5. Documentation

The data generated can be presented, if necessary, as a statement of precision and accuracy for a particular analysis on a given matrix. Record the percent recovery of the MS/MSD on the raw data or in the lab book. Enter the MS/MSD results in LABSYS2 into the MS/MSD entry.

7.8. Corrective Action.

If the method performance criteria outlined above cannot be met, notify your supervisor immediately.

7.9. Documentation.

All analytical data will be verified for completeness of QCI requirements, and will be spot checked for correct calculations. This verification will be performed by a competent analyst or the area supervisor.

All quality control data should be retained and available for easy reference and inspection.

8. REFERENCES

8.1. Methods for Chemical Analysis of Water and Wastes, USEPA Environmental Monitoring and Support Laboratory EPA-600/4-79-020 Revised March 1983.

8.2. Industrial Method No. 780-86T, Ammonia in Water and Wastewater, Bran & Luebbe Inc., Technicon Traacs 800 Method, Revised June 1987.

9. Daily Analytical Sequence

1. Calibration Curve: five point (per run) curve cc = > 0.995, calibration standards must be within 90-110% of true value except for the low standard, which must be within 80-120%.
2. Initial Calibration Verification Standard (ICVS): 90-110% of the true value or within the acceptance ranges established by the agency supplying the standard. Must be analyzed when a curve is run.
3. Reagent Blank (RB): < Reporting Limit or within the statistically established range, at the beginning, at the end, and 1 per every 10 samples.
4. Continuing Calibration Verification Standard (CCVS): 90-110% of the true value or within the statistically established range, every 10 samples and at the end of the run.
5. Matrix Spike/Matrix Spike Duplicate: 75-125% and an RPD less than 20 or within the statistically established range, 1 per every 20 samples.
6. Samples 1-10
7. CCVS
8. RB
9. If additional samples are to be analyzed, return to #6...

Always end the sequence with an RB and CCVS